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The effect of X-ray irradiation on *Salmonella* inactivation and sensory quality of almonds and walnuts as a function of water activity

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ABSTRACT

The overall goal of this study was to develop a set of process design principles for low-energy X-ray irradiation of tree nuts. Almonds and walnuts were inoculated with *Salmonella* Enteritidis PT30 and *Salmonella* Tennessee, and conditioned to four different water activities (0.23, 0.45, 0.64, and 0.84 a_w). Thereafter, the inoculated/conditioned samples were irradiated to achieve up to a 5-log reduction in *Salmonella* using a pilot scale low-energy X-ray food irradiator. Greater efficacy (D_{10}-value: the dose required to eliminate 90% of the microbial population) for inactivating SE PT30 and *S.* Tennessee was seen on the surface of almonds (0.226–0.431 kGy) than on walnuts (0.474–0.930 kGy) at all water activities. Also, the efficacy did not change monotonically with water activity. Overall, no significant difference ($P>0.05$) in sensory characteristics was seen between non-irradiated almonds and those irradiated to achieve a 5 log reduction in *Salmonella*. However, irradiating walnuts to the dose corresponding to a 5 log reduction caused a perceivable change in flavor. Post-irradiation storage tests revealed that surviving bacterial counts did not change over 120 days, regardless of nut type, *Salmonella* serovar, and a_w. Therefore, low-energy X-ray irradiation technology appears to be a promising non-thermal pasteurization strategy for certain types of nuts.

1. Introduction

California almonds were implicated in two outbreaks of salmonellosis in 2000 and 2003 that were traced to *Salmonella* Enteritidis PT30, prompting the recall of nearly six million kg of raw almonds (Anonymous, 2004; Isaacs et al., 2005; Keady et al., 2004) and the development of various pasteurization strategies for the industry. After the Almond Board of California proposed preventative measures, the final mandate calling for a minimum 4-log reduction of *Salmonella* on all California almonds was published in 2007 (USDA Agricultural Marketing Service, 2007). The fact that raw almonds were not previously pasteurized has created an urgent, industry-wide demand for technologies that can both achieve the mandated reduction in *Salmonella* and maintain the sensory and quality characteristics of the raw product. Consequently, various intervention technologies have been assessed, including propylene oxide fumigation (Danyluk et al., 2005), moist heating (Jeong et al., 2009), steam pasteurization (Chang et al., 2010; Sun-Young et al., 2006), acid spraying (Pao et al., 2006), hydrostatic pressure (Goodridge et al., 2006), water pressure (Willford et al., 2008), sanitizers, dry heat, hot water, and gas catalytic infrared heat (Latiful Bari et al., 2009), and irradiation (Mexis et al., 2009; Narvaiz et al., 1992; Prakash et al., 2010). Compared to hydro, thermal, and chemical methods, ionizing radiation has the advantage of retaining the functional quality of nuts. However, radiation efficacy varies among studies due to different sample conditions during treatment.

Although low water activity (a_w) is one key to controlling microbial growth, it actually presents a significant impediment to microbial inactivation. According to Laroche et al. (2005), the thermal inactivation rates for *Saccharomyces cerevisiae* and *Lactobacillus plantarum* are not monotonically dependent on initial a_w (0.10–0.70). Additionally, the effect of a_w on thermal resistance of *Salmonella* Typhimurium varied with solute type (glycerol, sucrose, glucose, or polyethylene glycol) (O'Donovan-Vaughan and Upton, 1999). Given that the surrounding humidity can alter the surface a_w of nuts during processing and storage, it is important to quantify inactivation rates as a function of this critical variable. Therefore, the objectives of this study were to: (1) quantify the relationship between a_w and the D_{10}-value for low-energy X-ray inactivation of *Salmonella* on almonds and walnuts, (2) quantify post-irradiation survival of *Salmonella* on nuts during storage, and (3) determine the impact of X-ray irradiation on the sensory quality.

2. Material and methods

2.1. Materials

Shelled raw whole almonds (Nonpareil) and walnuts (*Juglans regia*) from the 2009 crop were purchased in a single lot of each from retail sources located in California. Upon acquisition, 200 g of each nut type were vacuum-packaged and stored at 4 °C until testing. Kernel and bulk density were measured in a graduated cylinder using

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the platform scale method (AOAC 971.25) (AOAC, 2000). For moisture content determination, samples were ground using a IDS55 coffee bean grinder (Mr. Coffee, Cleveland, OH) for ~30 s, and ~2 g (5 replications) was dried in an oven at 102 °C to constant weight (~48 h). An FP-200 Nitrogen Analyzer (Leco Corp., St. Joseph, MI: compliance with AOAC 990.03) was used for protein analysis, and a Soxhlet fat extractor was used according to AOAC 948.22 (AOAC, 2000) for total fat content.

2.2. Salmonella strains and inoculation

Salmonella Enteriditis PT30 (SE PT30), originally isolated from raw almonds implicated in the 2000 and 2001 outbreaks, was previously obtained from Dr. Linda Harris (University of California — Davis) and preserved at −80 °C in Tryptic Soy Broth (TSB) (Difco, Becton Dickinson, Sparks, MD) containing 20% glycerol. To compare serovars, three strains of *Salmonella* Tennessee (S13952, S13972, and S13999) were obtained from the Food Science and Human Nutrition culture collection at Washington State University (Pullman, WS) and preserved under the same condition as SE PT30.

The inoculation procedure of Danyluk et al. (Danyluk et al., 2005) was followed with slight modifications. Before use, the culture was subjected to a minimum of two consecutive 24 h/35 °C transfers in TSB and then streaked to plates (150 mm × 15 mm) of Tryptic Soy Agar (Difco) supplemented with 0.6% yeast extract (TSAYE) to obtain a uniform lawn. After 24 h of incubation at 35 ± 2 °C, the bacterial lawn was harvested in 10 ml of sterile 0.1% peptone water (Difco), which was then added to 30 ml of 0.1% peptone water. Thereafter, 15 ml of culture was mixed with 150 g of each nut type in a sterile Whirlpak® filter bag for 1 min to give a target inoculum of ~10^8 CFU/g, after which the nuts were poured onto a raised aluminum mesh rack and dried in a biosafety hood at an air flow of ~0.56 m/s for 20 min to remove excess peptone water. Thereafter, the inoculated samples were transferred to a glove box (EW-34788-00, Cole-Parmer, Vernon Hills, IL) for subsequent water activity (a_w) conditioning.

2.3. Water activity conditioning

Four saturated salt solutions — CH_3COOK, K_2CO_3, $NaNO_2$, and KCl, were used to condition the nuts to a_w values of 0.23, 0.45, 0.64, and 0.84 at 20 °C, respectively. The lid of a steel tray was modified by installing a small fan and inlet/outlet holes to enhance air circulation inside the glove box. The tray was filled with 150–250 g of the appropriate salt and then saturated with de-ionized water. The conditioning salt tray, inoculated nut samples, a water activity meter (Hygrolab 3, Rotronic Instrument Corp., Hauppauge, NY), a digital relative humidity/temperature meter (pre-installed in the glove box), and Whirl-Pak® sample bags (4 oz) (Nasco, Fort Atkinson, WI) were then placed in the glove box, after which the main door was closed for further conditioning. To monitor the conditioning process, tightly sealed Petri dishes (10 mm × 40 mm diam.) containing ~10 g of each nut type were removed from the glove box through a pass box door that maintained a closed system for the sample and the glove box. Conditioning to equilibrium moisture content (EMC) (<0.03% weight change over ~24 h) usually took about 6–7 days. After reaching equilibrium, ~5 g of the conditioned nuts was transferred to a sterile Whirl-Pak® sample bag in the conditioning glove box, in order to maintain the established humidity around the sample. Final EMC was measured using an oven drying method, and a_w was measured using the water activity meter on the day of irradiation.

2.4. Irradiation

The inoculated a_w-conditioned samples (5 g, ~5 nuts) were irradiated in a prototype X-ray irradiator (Rainbow™ II, Rayfresh Foods Inc., Ann Arbor, MI), which currently is housed in the biosafety level-2 pilot plant at Michigan State University. The irradiator consists of an industrial grade X-ray tube (modified OEG-75, Varian Medical System, Salt Lake City, UT), high voltage source, and cooling unit. The X-ray tube operates at a maximum constant potential of 70 kV and a filament current of 57 mA, which gives 4 kW of maximum allowable input power. Five different surface doses (0.3–5.5 kGy) of X-ray radiation at maximum power (70 kV/57 mA) were applied to achieve 1 to 5 log reductions at each a_w condition and nut type, with all experiments conducted in triplicate. Each sample was irradiated on both sides by flipping the sample halfway through treatment, to achieve a uniform dose. The typical dose rate was ~20 Gy/s.

2.5. Dosimetry

Nominal surface dose was measured using radiochromic film dosimeters (GAF3001DS, GEX Corporation, Centennial, CO). At each a_w level, dose rate at the bottom of the nut was measured to calculate total accumulated dose in the double treatment configuration. The a_w-conditioned nuts were placed on top of the dosimeter and exposed to the X-ray radiation for 125 s to obtain a measurable dose on the dosimeter. For each a_w and nut type, 3–5 samples were used to measure the dose rate at the bottom, with this dose information then used to estimate total dose on the nut surface. The dose rate at the bottom was ~10% of the top surface dose rate. The sum of the dose rates at the top and bottom was used to estimate total accumulated surface dose. The dosimeters were read 24 h after irradiation using a standard spectrophotometric method (Spectronic Genesys 20, Thermo Fisher Scientific, Inc., Waltham, MA) based on calibration curves at 500/550 nm.

2.6. Salmonella enumeration

Following irradiation, 45 ml of sterile 0.1% peptone was added to the treated bags containing 5 g of nuts. Samples were massaged by hand for 1 min and then homogenized in a Stomacher (Masticator, Neutec Group Inc., Farmingdale, NY) for 3 min. Appropriate serial dilutions were surface-plated on TSAYE supplemented with ferric ammonium citrate (0.05%) and sodium thiosulfate (0.03%) to differentiate colonies of *Salmonella* (characteristic black precipitate in the center) from those formed by any background microorganisms. The plates were incubated at 35 ± 2 °C for 48 h. As is standard for analysis of pasteurization processes, the outcomes were first converted to log reductions, which were calculated by subtracting the log of the survivor counts for each individual observation from the mean log counts on inoculated, untreated samples. The D_{10}-values (i.e., the inverse of the slope between the applied X-ray dose and log reduction) were then determined by linear regression.

2.7. Sensory evaluation test

Initially, a triangle test was used to determine any overall sensory difference between irradiated and non-irradiated almonds and walnuts. Almonds (steam pasteurized) and walnuts for tests were purchased seven days before sensory analyses and tested for the absence of countable *Salmonella* as previously described. Individual bags containing ~5 g each of almonds and walnuts were irradiated (outside of the BSL-2 pilot plant) at 1.13 and 2.37 kGy, respectively, which were the lowest effective doses able to achieve a 5 log reduction for *Salmonella*. The bagged nuts were then held in stainless steel canisters no longer than two days at room temperature until testing.

Michigan State University students, staff, and faculty who are consumers of almonds and walnuts were recruited to participate in a sensory test comparing non-inoculated, low-energy X-ray treated nuts to a non-inoculated, non-irradiated control. The tests were conducted in the Department of Food Science and Human Nutrition's sensory evaluation laboratory, which includes seven panelist booths, controlled lighting, and computers equipped with Sensory Information Systems (SIMS version 6.0 software for data collection).

For the triangle test, randomized three digit numbers were used to label X-ray treated and control almond and walnut samples. The

control sample was the odd sample in half of the tests, and the treated sample was the odd sample in the other half. After being instructed on how to evaluate the samples using an example tray and test demonstration, panelists were asked to pick the sample that differed from the other two in the almond triangle test. Panelists then were presented the walnut samples using the same protocol.

If panelists could detect an overall difference between the control and test sample, an acceptance test would determine if this difference significantly affected consumer acceptability. Based on the triangle tests results, an acceptance test was run for the walnut control and irradiated samples to evaluate appearance, aroma, flavor, texture, and overall acceptability. The same dose that was used for the difference test was applied to the nuts, and the acceptance test was conducted with 75 nut consumers. A nine point hedonic scale was used with 9 = "like extremely," "8 = like very much," "7 = like moderately," 6 = like slightly, "5 = neither like nor dislike," "4 = dislike slightly," "3 = dislike moderately," "2 = dislike very much," "1 = dislike extremely."

2.8. Post-irradiation survival test

To investigate the fate of *Salmonella* during long-term storage, nuts were inoculated, irradiated, and held at 4 °C/70%RH. Almonds and walnuts were inoculated with SE PT30 and conditioned at 0.2 and 0.7 a_w. Thereafter, the inoculated samples were bagged inside the conditioning chamber to maintain the established a_w and irradiated at doses of 1.13 (almond; SE PT30; 0.2 a_w), 2.37 (walnut; SE PT30; 0.2 a_w), 2.28 (almond; SE PT30; 0.7 a_w), 4.32 (walnut; SE PT30; 0.7 a_w), 2.28 (almond; *S.* Tenn.; 0.7 a_w), and 4.32 (walnut; *S.* Tenn.; 0.7 a_w) kGy to achieve ~5 log reductions (not the presence/absence test) at the corresponding a_w values. Therefore, plate counts were always positive and there was no need of enrichment process.

The bags of SE PT30 inoculated nuts were opened and placed in a refrigerator at 4 °C/~70%RH for 7 days to equilibrate with the storage conditions. Thereafter, the bags were closed to prevent any further contamination and returned to storage for up to 120 days. In contrast, the bags of *S.* Tennessee-inoculated nuts were previously conditioned to 0.7 a_w and consequently remained closed during storage. Three bags each of the irradiated and control nuts were randomly selected after 1, 30, 60, 90, and 120 days of storage and quantitatively examined for *Salmonella* as previously described.

2.9. Statistical analysis

Linear regression analysis was applied to the dose-survivor data after confirming normality of the data using the Anderson–Darling normality test, via Minitab 15 (Minitab Inc., State College, PA). The 95% confidence intervals of the D_{10}-value were also computed. To test the difference in D_{10}-values between almond and walnut, regression lines were compared using analysis of covariance (ANCOVA) via Minitab 15. Post-irradiation storage data were subjected to ANOVA and linear regression to test for any changes with storage time. ANOVA and Tukey's honestly significant difference test (SAS® 9.1, SAS Institute Inc., Cary, NC) were used to evaluate the sensory data.

3. Results and discussion

3.1. Proximate analyses

Physical and compositional characteristics of the raw almonds and walnuts, measured before any treatment (Table 1), were consistent with published data (Agricultural Research Service, 2010; Sze-Tao and Sathe, 2000). Corresponding EMCs for target water activities for almond and walnut (Table 2) also were consistent with published sorption isotherm data for nuts (King et al., 1983; Pahlevanzadeh and Yazdani, 2005; Togrul and Arslan, 2007).

Table 1
Physical properties and proximate composition of almonds (Nonpareil) and California walnuts.

Properties	Almond	Walnut
Kernel density [g/cm^3]	1.04 ± 0.01	0.96 ± 0.01
Bulk density [kg/m^3]	611	411
Moisture content [% d.b.]	5.12–5.84	3.11–3.42
Protein content [% w.b.]	25.68	17.92
Fat content [% w.b.]	48.72–51.90	62.62–65.25

3.2. Microbial efficacy of X-ray irradiation based on nut type

An initial inoculation level of 8.40 ± 0.14 log CFU/g (n = 12) on almonds and 8.65 ± 0.23 log CFU/g (n = 12) on walnuts was achieved for SE PT30. For *S.* Tennessee, an inoculation level of 7.73 ± 0.32 (n = 12) and 7.87 ± 0.25 log CFU/g (n = 12) was achieved for almonds and walnuts, respectively. Based on the log reductions vs. total accumulated surface dose at the various a_w levels, both SE PT30 and *S.* Tennessee were more resistant on walnuts than on almonds (Figs. 1 and 2). Despite the inherent difficulty in accurately measuring surface dose on almonds and walnuts, the inactivation curves for SE PT30 on almonds and walnuts yielded statistically different slopes ($\alpha = 0.05$), based on the ANCOVA (Table 3), reaffirming different efficacies between the two nut types.

3.3. Microbial efficacy of X-ray irradiation based on water activity

The status of water in/around microorganisms is an important factor determining the efficacy of any lethal agent. The sorption isotherms (a_w vs. equilibrium moisture content; Table 2) showed a moderately controlled adsorption process, with a 0.16 and 0.84% (d.b.) mean difference between the experimental EMC and Guggenheim–Anderson–deBoer (GAB) model predictions for almonds (Pahlevanzadeh and Yazdani, 2005) and walnuts (Togrul and Arslan, 2007), respectively.

Sensitivity of the D_{10}-value to water activity (Fig. 3) is a critical design factor to consider when irradiating low water activity foods. Based on our results, SE PT30 was less resistant to irradiation at the two lowest as compared to the highest two water activities, with maximum resistance seen at 0.6–0.7 a_w. It is often suggested that *Salmonella* becomes more resistant to lethal agents as the water activity of a food product is lowered (Aldsworth et al., 1998; Archer et al., 1998; Carlson et al., 2005; Shadbolt et al., 2001). However, this was only true at higher water activities (Black and Jaczynski, 2008), as Fig. 3 illustrates that the relationship between resistance and a_w is not monotonic.

Table 2
Water activity and corresponding equilibrium moisture contents (EMC) for almonds and walnuts inoculated with *Salmonella* Enteritidis PT30 and *Salmonella* Tennessee.

Product	*Salmonella* serotype	a_w	EMC [% d.b.]	Temperature[b] [°C]
Almond	*S.* Enteritidis PT30	0.226	n/a[a]	20.38
		0.445	3.92	22.06
		0.637	7.96	21.46
		0.841	n/a[a]	21.01
	S. Tennessee	0.207	5.37	20.89
		0.410	5.86	20.85
		0.607	8.17	20.75
		0.804	12.17	20.91
Walnut	*S.* Enteritidis PT30	0.227	n/a[a]	20.40
		0.445	1.72	21.34
		0.646	3.90	20.57
		0.845	n/a[a]	21.48
	S. Tennessee	0.200	2.40	21.59
		0.383	3.43	21.84
		0.583	3.52	21.12
		0.785	5.53	20.98

[a] No sample taken.
[b] Temperature when a_w was measured.

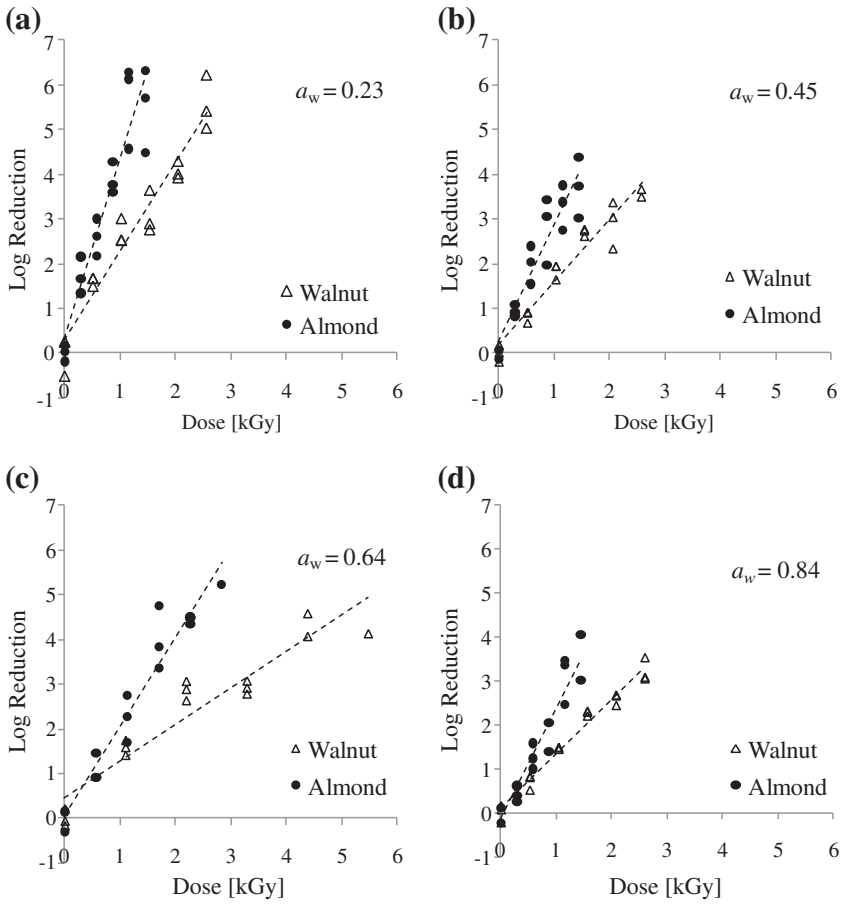

Fig. 1. Inactivation of *Salmonella* Enteritidis PT30 via low-energy X-ray irradiation on surface-inoculated almonds and walnuts at various water activities.

Given the similar D_{10}-values for SE PT30 and *S.* Tennessee (Table 3), Fig. 3 suggests that the D_{10}-value is a function of water activity and product type rather than the serovar of *Salmonella*. Based on these findings, *Salmonella* Enteritidis PT30 and *S.* Tennessee were less resistant to irradiation on surface-inoculated almonds and walnuts when the nuts were in their driest state, which conflicts with the study (Thayer et al., 2003) in which alfalfa seed size, water activity, and moisture did not significantly affect the D_{10}-value.

The underlying causes are likely related to several critical factors, including water activity (Black and Jaczynski, 2008), temperature (Black and Jaczynski, 2006), dose rate, the extent of direct (DNA damage) / indirect (free radicals) (Kwakwa and Prakash, 2006; Molins, 2001) radiation absorbance by water (Bierman et al., 1956), the physiological state of the microorganisms, and favorable/unfavorable microbial byproducts (Barbosa-Cánovas et al., 2007). However, radiation sensitivity (D_{10}-value) for *Salmonella* Typhimurium inoculated on various seeds (green gram, dew gram, chick pea, and garden pea) was found to vary significantly (Saroj et al., 2006), which implies that multiple nonlinear factors may result in the D_{10}-value pattern in Fig. 3. While numerous studies have assessed the efficacy of ionizing radiation, most of these studies used high-energy rather than low-energy radiation and did not specify water activity (Hvizdzak et al., 2010; Mexis and Kontominas, 2009a, 2009b; Prakash et al., 2010). To our knowledge, this is the first study to directly assess the impact of water activity on the efficacy of low-energy irradiation on dry product, with our findings being consistent for two *Salmonella* serovars and two nut types. Overall, the results of this study indicate that low-energy X-ray is a viable non-thermal alternative for nut pasteurization, but that the process, and impact on quality, are clearly product-specific.

3.4. Sensory evaluation

A total of 67 panelists completed the triangle test, with 43 females and 24 males participating. The panelists ranged in age from 18 to 60 and older. The most represented ages were 25–34 and 18–24, with 23 and 21 panelists, respectively. Overall, 64.2% of the panelists reported consuming nuts once a week or more, and none of the panelists consumed nuts less than once a month. Responses in terms of the type of nuts consumed, 89.6% of the panelists reported consuming almonds, followed by peanuts (82.1%), walnuts (74.6%), cashews (67.2%), pistachios (44.8%), and other nuts (26.9%).

Twenty-three of 67 (34.3%) panelists selected the correct almond sample in the triangle test, indicating no significant difference between samples ($P<0.05$). For the walnut triangle test, 44 of 67 (65.7%) panelists correctly chose the walnut sample that was different from the other two samples, which was statistically significant ($P=0.001$). Panelists detected an off flavor or "fishy taste" related to oxidative rancidity in the irradiated walnut samples in subsequent acceptance testing of irradiated walnuts. Eighty four percent of the 75 consumers (28 males and 47 females) who participated in the test consumed nuts several times a month or more. No significant differences were seen in appearance or aroma. However, significantly lower scores were found in texture, flavor, and overall acceptability, with flavor being the most likely contributor to the lower overall score for walnuts irradiated at 2.37 kGy (Table 4). Mexis and Kontominas (2009a, 2009b) evaluated the physicochemical and sensory attributes of walnuts as a function of gamma irradiation dose. They found a significant increase in peroxide value (PV) and hexanal content compared to the control when walnuts were irradiated at 1.0,

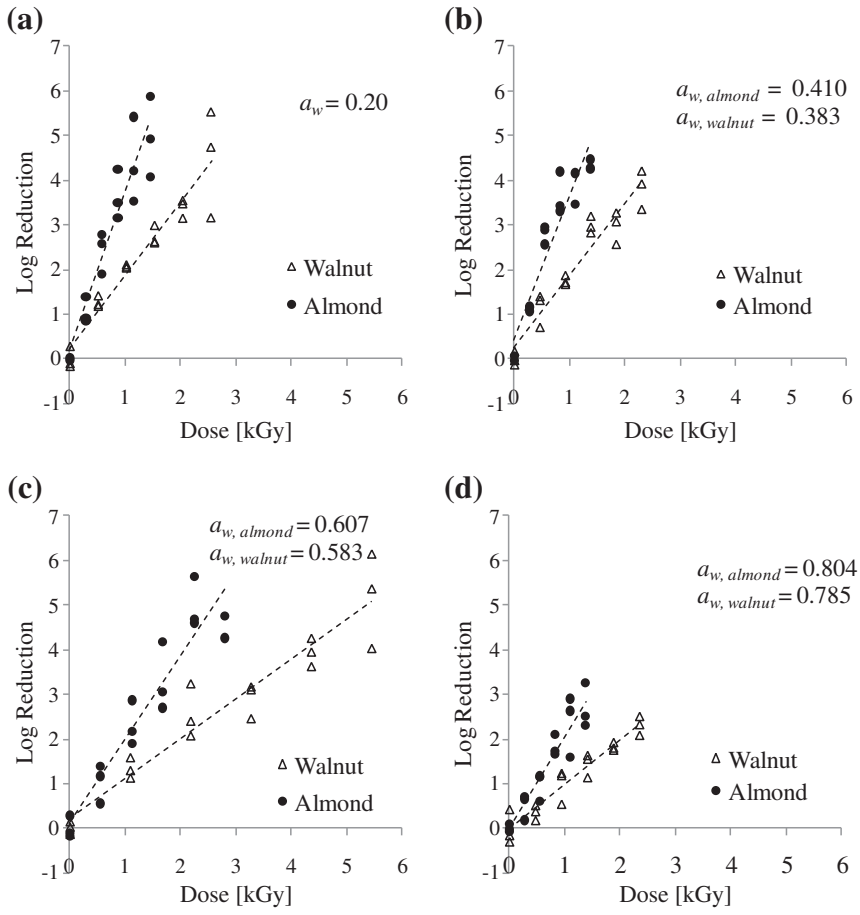

Fig. 2. Inactivation of *Salmonella* Tennessee via low-energy X-ray irradiation on surface-inoculated almonds and walnuts at various water activities.

1.5, 3.0, 5 and 7.0 kGy. Sensory testing resulted in significantly lower taste acceptance for each increase in radiation dose. Since irradiation can generate free radical formation resulting in lipid oxidation, these results can be expected (Sajilata and Singhal, 2006). However variations in fatty acid composition of the nuts, radiation doses and naturally occurring antioxidants in the nuts can cause some differences in values. No significant differences in flavor were found by untrained panelists using triangle tests when almonds were irradiated at 1 kGy. In a more recent study, Mexis et al. (2009) found significant increases in PV of almonds and significant decreases in flavor sensory acceptability as doses increased (1.0, 1.5, 3, 5, 7 kGy). However, a dose of 1.0 kGy resulted in a score of 8.0/9.0, indicating "like very much," still highly acceptable. The authors concluded that doses up to 3 kGy did not adversely affect the almond sensory quality. Sanchez-Bel et al. (2005) conducted trained sensory evaluation of almond quality after electron beam irradiated almonds. After irradiation and 121 days storage, no significant differences in rancidity and overall quality compared to the control were found for almonds irradiated at 1, 3 or 7 kGy. Prakash et al. (2010) found significant decrease in the flavor and overall quality when almonds were irradiated at 2.98 and 5.25 kGy, higher than used in the current study. These research study results support why no significant differences were detected in the triangle test conducted in the current study for almonds (1.13 kGy) while significant differences were found for walnuts (2.37 kGy). Walnuts were subjected to a higher

Table 3
D_{10}-value for low-energy X-ray inactivation of *Salmonella* Enteritidis PT30 and *Salmonella* Tennessee on the surface of almonds and walnuts at various water activity levels.

Salmonella serotype	a_w[1]	D_{10}-value ± C.I.[2] [kGy]	
		Almond	Walnut
S. Enteritidis PT30	0.2	0.226 ± 0.039^a	0.474 ± 0.062^b
	0.4	0.338 ± 0.065^a	0.669 ± 0.094^b
	0.6	0.471 ± 0.068^a	1.092 ± 0.223^b
	0.8	0.363 ± 0.059^a	0.785 ± 0.069^b
S. Tennessee	0.2	0.256 ± 0.044^a	0.554 ± 0.093^b
	0.4	0.282 ± 0.052^a	0.577 ± 0.088^b
	0.6	0.479 ± 0.090^a	1.029 ± 0.166^b
	0.8	0.431 ± 0.082^a	0.930 ± 0.141^b

Values with different lowercase letters within rows indicate a significant ($P<0.05$) difference in efficacy due to nut type.
[1] Nominal surface a_w.
[2] 95% confidence level.

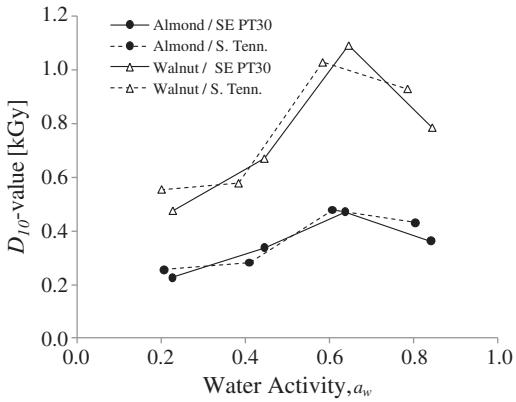

Fig. 3. Relationship between water activity and the D_{10}-value for low-energy X-ray inactivation of *Salmonella* Enteritidis PT30 and *Salmonella* Tennessee on surface-inoculated almonds and walnuts.

Table 4
Means of the acceptance sensory scores testing the control and walnuts irradiated at 2.37 kGy using low-energy X-ray (9 point hedonic scale, 9 = like extremely, n = 75).

Attribute	Control walnuts	Irradiated walnuts
Appearance	7.07[a]	7.03[a]
Aroma	5.81[a]	5.79[a]
Flavor	7.28[a]	5.89[b]
Texture	7.31[a]	7.03[b]
Overall	7.27[a]	6.09[b]

Values with different lowercase letters within rows indicate significant ($P<0.05$) difference in attribute between control and irradiated sample.

X-ray radiation dose and have been shown in the literature to be more susceptible to decreases in flavor scores with increasing irradiation.

3.5. Post-irradiation survival test

Initial populations of 7.47 ± 0.52 and 8.05 ± 0.36 log CFU/g for almonds and walnuts were achieved, respectively. For corresponding tests with *S.* Tennessee, samples were conditioned at 0.7 a_w, resulting in initial populations of 7.73 ± 0.22 and 8.32 ± 0.52 log CFU/g for almonds and walnuts, respectively. *Salmonella* populations were quantifiable on all positive control and irradiated almonds and walnuts up to 120 days (Fig. 4) with no significant ($\alpha = 0.05$) increase or decrease in numbers of salmonellae during storage. Regression analysis yielded

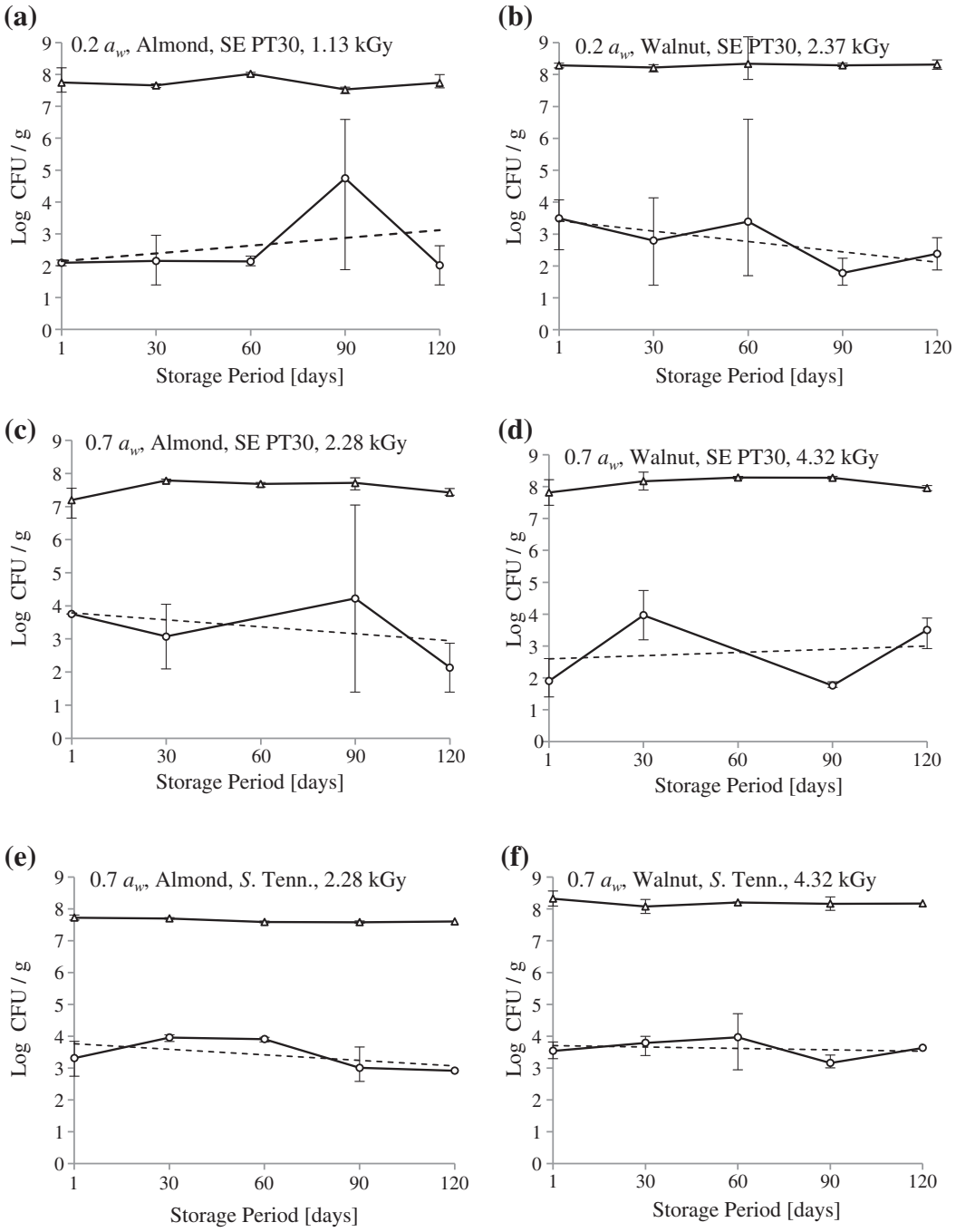

Fig. 4. Survival of *Salmonella* Enteritidis PT30 and *Salmonella* Tennessee on control and irradiated surface-inoculated almonds and walnuts during storage at 4 °C as impacted by water activity. (The error bars represent maximum and minimum values). -△- Positive control; -○- irradiated sample; ----- linear regression (irradiated sample).

high *P*-values (0.1727–0.7992) against the slope, with no significant relationship seen between the numbers of salmonellae and storage period. Uesugi et al. (2006) also failed to see a significant reduction in numbers of *Salmonella* during 550 days of storage at −20 and 4 °C, which supports our findings during 120 days of storage with no significant sublethal effect of irradiation seen on the survivors.

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